Nicotine Regulates *Streptococcus mutans* Extracellular Polysaccharide and Related Protein Expression.

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Streptococcus mutans, a gram-positive facultatively anaerobic bacterium, is considered as the primary contributor to caries due to its high acidogenicity and aciduricity. Smoking is one of the risk factors of periodontal disease and dental caries. Nicotine is one of the alkaloid pharmacologically active agents in tobacco. Previous studies indicated nicotine stimulated *S. mutans* biofilm formation and metabolism. However, the detailed mechanism is still unknown. Thus, the aim of this study is to investigate how nicotine facilitates *S. mutans* biofilm formation focused on extracellular polysaccharide synthesis. *S. mutans* UA159 (ATCC 700610) was used in the present study. Confocal laser scanning microscopy (CLSM) was used to investigate the effect of 0, 1, 2 and 4 mg/ml nicotine on 24 h *S. mutans* biofilm extracellular polysaccharide (EPS) expression (red fluorescent-labeled) and nucleic acid expression (green fluorescent-labeled). Western blot assays were used to investigate the effect of 0, 1, 2 and 4 mg/ml nicotine on the expression of glucosyltransferase (Gtfs), glucan-binding protein A (Gbp-A) and Gbp-B in 24 h *S. mutans* biofilm cells. CLSM results indicated nicotine increased both EPS and nucleic acid, and the ratio of EPS/nucleic acid was also increased. It implied EPS synthesis in single *S. mutans* cells was stimulated by nicotine treatment. Biofilm thickness was thicker in nicotine-treated groups than the non-treated group. Western blot assay results indicated that nicotine stimulated GtfC, Gbp-A and Gbp-B expression, but decreased GtfB expression. In conclusion, nicotine stimulates *S. mutans* cell proliferation and EPS synthesis partially by increasing GtfC, Gbp-A and Gbp-B.

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